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Antivenom activity of opossum (Didelphys marsupialis) serum fractions against Uracoan rattlesnake (Crotalus vegrandis Klauber, 1941) venom.
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ANTIVENOM ACTIVITY OF OPOSSUM (*Didelphys marsupialis*) SERUM FRACTIONS AGAINST URACOAN RATTLESNAKE (*Crotalus vegrandis* KLAUBER, 1941) VENOM

by

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In this work, we have found strong evidence for the presence of an opossum serum which is highly proficient in inactivating the neurotoxic fractions of Uracoan rattlesnake (*Crotalus vegrandis*) venom. Analyses of strained electrophoretic patterns of SDS gels run in non-reducing conditions revealed a small group of antivenom proteins in 0.1 M DEAE cellulose fraction that was not found in 0.05 M, 0.02 M, 0.25 M and 0.3 M NaCl ionic strengh. Neutralizing activities to mapanare (*Bothrops lanceolatus*) venom have been already described but this is the first time that opossum serum anticrotalus activity is found. In spite of having preliminary results, we wish to make the corresponding report, while we accomplish the purification of the neutralizing component. One protein isolated from opossum serum or a synthetic peptide based on the aforementioned protein would probably be very useful in medical management of *Crotalus vegrandis* accidents.

Uracoan rattlesnake (*Crotalus vegrandis*) (Figure 1) is a *Crotalidae* present in a small geographic region of northeastern Venezuela, whose venom is a complex mixture of toxins and enzymes with biological effects of neurotoxic and myotoxic activities, such as respiratory paralysis, autonomic disturbances as salivation and flaccid or spastic paralysis of the posterior limbs /2,10,11/.

The observation, about the opossum (*Didelphis marsupialis*) resistance to the bite of some snakes, that it is fed in the nature, outlined the possibility that the marsupial has a natural immunity ability, that protects it against (*Crotalidae*)

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snake venoms with which come sharing, since thousands of years, the same ecological niche. Previous studies /6,9/ have demonstrated the neutralizing action of the Mr 97 kDa opossum serum fraction, against the *Bothrops* venom. The main aim of this work is to demonstrate the opossum serum inactivation activity on neurotoxic action of the Uracoan rattlesnake (*Crotalus vegrandis*) venom.

Figure 1 – Adult female *Crotalus vegrandis* Klauber, 1941 (Uracoan rattlesnake) showing the typical white spots on ash–colored dark background

MATERIALS and METHODS

Animals

In the neurotoxic test mice NIH strain weighing 18–22 g were used.

Serum

Blood was obtained by cardiac puncture of 10 opossum (*Didelphis marsupialis*) captured in Caracas valley and kept in the Tropical Medicine Institute Animal House, kept for 3 hours at 5°C and then centrifuged. The sera pooled and ammonium sulphate

(37% v/v) precipitated. The supernatant dialized against 0.01 M phosphate buffer saline (PBS) pH 7.2, dispensed in vials and stored at −30°C until used.

Venom

Venom from 12 specimens of *Crotalus vegrandis* captured near Uracoa, Monagas State (Venezuela) was milked, pooled and centrifuged at 2000 x G to remove cellular debris, and the supernatant lyophilized and stored at −70°C.

Lethal dose 50 (LD_{50})

LD_{50} was determined by inoculating intramuscularly (i.m.) *Cratalus vegrandis* venom, in 4 batches of 8 mice, with equivalent concentrations to 5, 10, 15 and 20 mg/kg of mouse body weight in each batch.

Protein determination

The method of Lowry was used.

Ion exchange chromatography

Immunoglobulins free opossum serum was bound DEAE–cellulose columns. The proteins were eluted by solutions with increased ionic strengh (0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M and 0.3 M). Fractions were concentrated by vacuum dialysis, dispensed in vials and stored at −30°C.

Toxicity determination

The dosage of venom injected in each inactivation test was standardized according to the method used by Rodriguez–Acosta and Aguilar (1987) /8/. Briefly, the lethal toxicity was assessed by intramuscular injection of the venom (0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg and 0.8 mg) in 0.1 ml of saline into mice. Eight animals were used at each venom dose and the Lethal Dose Fifty (LD_{50}) was calculated by probit analysis /1/ of deaths occuring within 24 hrs of venom injection.

Neurotoxic activity

This activity was tested by *in vivo* experimental assay, inoculating (i.m.) 6 mice with a LD_{50}.

Inactivation of neurotoxic venom effects

A constant amount (15 mg/kg) of total venom was mixed with different amounts (5 mg, 10 mg, 15 mg, 20 mg, 25 mg) of opossum immunoglobulins–free serum proteins and incubated for 30 minutes at 37°C and injected (i.m.) into mice, to carried out, including positive controls, *in vivo* antineurotoxic activity determination.

Different amounts of each opossum serum fractions were tested with a contant amount (LD_{50}) of venom and used as follows: each opossum serum fraction (15 mg protein) and crude venom (15 mg/kg) were mixed and incubated for 30 minutes at 37°C and then injected (i.m.) into mice, to test *in vivo* antineurotoxic activity determination.

RESULTS

LD_{50}

It was calculated of 15 mg/kg of body weight. As expected, the most characteristic symptoms were convulsions, paralysis of the posterior limbs and respiratory muscles.

Capacity of opossum serum to inactivate neurotoxic activity of Crotalus vegrandis venom

The serum of opossum inactivated the *Crotalus* venom neurotoxicity, but when heated serum was used, the neurotoxicity was not neutralized and mice died. The protective activity of the opossum serum supernatant, was recovered in the fraction eluted from an ion exchange column with 0.1 M NaCl (Table 1).

Table 1 – Capacity of total serum; heated serum and different fractions of opossum (*Didelphis marsupialis*) serum obtained by DEAE–cellulose to inactive neurotoxic activity of Uracoan rattlesnake (*Crotalus vegrandis*) venom

	Total serum*	Heated serum	DEAE–cellulose fractions			
			0.05 M	0.1 M**	0.15 M	0.2 M
Antineurotoxic activity***	+	–	–	+	–	–

The plus signs represent antineurotoxic activity. The minus signs indicate that the serum did not inactivate venom activities.

* Sulphate ammonium precipitated. 15 mg of serum supernatant inactivated 15 mg/ml of crude venom.

** 1 mg of serum fraction (0.1 M) inactivated 15 mg/kg of crude venom.

*** Specific (relative) inhibitory activity of opossum serum and its fractions is not shown, because the test only measured if neurotoxic symptoms were present or not.

Opossum immunoglobulins were unable to protect mice against *Crotalus* venom, only the ammonium sulphate treated supernatant of serum protected them against LD_{50} crude venom.

DISCUSSION

When serum and venom were incubated prior to the injection into mice, the serum of opossum inactivated the local and general effects of *Crotalus vegrandis* venom. A highly potent neurotoxin, crotoxin, is contained in this venom /2/; when injected into mice produces flaccid paralysis of respiratory muscles and death. The mechanism of the factor in opossum serum, which inactivates venom toxins is unknown and it may represent a natural immunity phenomenon. Heated serum showed that the factor involved in venom inactivation is labile at 56°C.

Authors (PIFANO, 1959 – personal communication) /4–7, 12/ have found that sera from warm-blooded animals can neutralize the activity of snake venoms.

Other authors /9/ have demonstrated a Mr 97 kDa protein from opossum (*Didelphis marsupialis*) which is capable to neutraliza *Bothrops lanceolatus* snake venom, but in the present paper it is for the first time that an anticrotalic activity in the opossum serum has been demonstrated. The present study provides strong eficience for the presence of an opossum serum molecule(s), which can inactivate neurotoxic effects of *Crotalus* venom. The inactivating capacity of the 0.1 M NaCl ion exchange fraction obtained is remarkable since 1 mg neutralizes 1 mg of venom. We propose that this antivenom, once we can isolate and purify the molecule, would be useful and safe for medical treatment of *Crotalus vegrandis* snakebite.

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